PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68 — A3

(11) International Publication Number: WO 00/28081

(43) International Publication Date: 18 May 2000 (18.05.00)

(21) International Application Number: PCT/IB99/01958

(22) International Filing Date: 9 November 1999 (09.11.99)

(30) Priority Data:
60/107,293 — 9 November 1998 (09.11.98) — US

(71) Applicant *(for all designated States except US)*: METHEXIS N.V. [BE/BE]; Onafhankelijkheidslaan 38, B–9000 Gent (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: ZABEAU, Marc [BE/BE]; Onafhankelijkheidslaan 38, B–9000 Gent (BE). STANSSENS, Patrick [BE/BE]; Constant Permekelaan 48, B–9830 St. Martens Laten (BE).

(74) Agent: DE CLERCQ, Ann; Ann De Clercq & Co B.V.B.A., Brandstraat 100, B–9830 Sint–Martens–Latem (BE).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
3 August 2000 (03.08.00)

(54) Title: RESTRICTED AMPLICON ANALYSIS

(57) Abstract

The present invention generally provides a method which facilitates the detection of polymorphisms (or mutations). The method is directed to the analysis of so–called endonuclease site polymorphisms (ESPs) that result in the gain or loss of a restriction endonuclease site. In essence, the ESP is probed with the restriction endonuclease reagent prior to amplification, whereby amplification is prevented and consequently no signal is observed when cleavage takes place. Unambiguous allele calling is performed by comparing the signals obtained with and without cleavage with the restriction endonuclease reagent. The method is particularly useful for multiplex genotyping, involving the parallel analysis of large numbers of single nucleotide polymorphisms. Preferred methods for detecting the amplicons involve hybridization to an arrayed or otherwise identifiable set of cognate probe fragments or oligonucleotides.